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TOXICITY OF CS-2 DECONTAMINATION PRODUCTS

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NON-EXPLOSIVES MUNITIONS DIVISION

TECHNICAL REPORT AFATL-TR-70-68

JULY 1970

AIR FORCE ARMAMENT LABORATORY
AIR FORCE SYSTEMS COMMAND • UNITED STATES AIR FORCE
EGLIN AIR FORCE BASE, FLORIDA

Toxicity of CS-2 Decontamination Products

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FOREWORD

This report documents research accomplished by the Assessments Branch, Non-Explosives Munitions Division, Air Force Armament Laboratory, and statistical analyses accomplished by Booz-Allen Applied Research, Inc. during the period July 1969 to June 1970. The active USAF project directly related to the information discussed herein is Engineering Development Project 2534.

The animals used in this project were cared for in the manner promulgated by the "Guide for Laboratory Animal Facilities and Care," and Public Law 89-544, "Laboratory Animal Welfare Act," 24 August 1966.

Use of trade names is for identification purposes only and does not constitute endorsement by the United States Air Force.

The statistical analyses by Booz-Allen Applied Research are gratefully acknowledged.

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This report has been reviewed and is approved.

JOHN E. HICKS, Colonel, USAF
Chief, Non-Explosive Munitions Division

ABSTRACT

The phytotoxicity and animal toxicity of decontaminated CS-2 and its hydrolysis products were determined in greenhouse and laboratory investigations. The acute oral lethal dose of CS-2 decontamination products for 50 percent of a rat population was established at 1.68 g/kg. For a 96-hour exposure period, the median tolerance limit values for decontaminated CS-2 were 18.95 ppm for mosquitofish and 16.78 ppm for bluegill. During three generations of Swiss Webster mice receiving 500 ppm decontaminated CS-2 in their drinking water, there were no detectable abnormalities in the offspring. Decontaminated CS-2, as a soil drench, was found to be toxic to plants when it was applied at the rate of 100 ml of a two percent solution per four-inch pot. No damage occurred to plants that received equal quantities of a 0.25 percent solution. The phytotoxicity of decontaminated CS-2 is thought to be caused by its hydrolysis products, o-chlorobenzaldehyde and malononitrile. Plants treated with decontaminated CS-2 and with equivalent amounts of these hydrolysis products all exhibited the same degree of damage.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SECTION I

INTRODUCTION

The objective of this project was to establish the precautions that should be observed during the disposal of o-chlorobenzalmalononitrile, riot control agent CS, after neutralization with a 3:1 monoethanolamine-water decontaminating solution and a 1:1 2-propanol-water wetting solution.[1]

The research reported in this study deals with determination of fish, plant, and mammalian toxicities of the products of CS-2 decontamination.

SECTION II

TEST PROCEDURES

Investigations for this project were conducted in three parts: (1) greenhouse studies with plants, (2) laboratory studies with mammals, and (3) laboratory studies with fish. The procedure for decontaminating CS-2 was identical for each study. A 2-propanol-water (50:50 v/v) mixture was added to wet the CS-2 (1.67 ml 2-propanol-water/g CS-2). The decontaminating solution, monoethanolamine-water (75:25 v/v mixture), was then added (1.0 ml/g CS-2) and mixed uniformly. All CS-2 used in these investigations was decontaminated 16 to 20 hours prior to use in the individual experiment.

Plant Studies

The phytotoxicity data were determined by a 4 x 6 x 5 experiment in randomized block design, replicated four times. An experimental unit consisted of a four-inch pot containing three-week-old plants. Blocks consisted of four plant species, cotton (Gossypium hirsutum L. var. Stoneville 213), tomatoes (Lycopersicon esculentum Mill. var. Homestead 24), ryegrass (Lolium temulentum L. Darnel), and rice (Oryza sativa L. var. Nato). Plants were grown in a clear glass greenhouse with minimum night temperatures of 18 C and maximum day temperatures of 35 C. The potting medium was formulated by combining six cubic feet of perlite, four pounds of dolomite lime and one pound of superphosphate with 13 cubic feet of steam-sterilized sandy loam. Each pot contained approximately 400 grams of potting soil. Plants were fertilized with liquid 15-15-15 prior to treatment. The plants were treated with 100 ml soil applications of water containing various concentrations of o-chlorobenzaldehyde, malononitrile, monoethanolamine, 2-propanol, 2-propanol-monoethanolamine, and decontaminated CS-2, respectively.

The concentrations of o-chlorobenzaldehyde and malononitrile used were based on the determination of the quantities of the two compounds that would be formed from complete hudrolysis of CS-2. Actual quantities used are shown in Table 1. A surfactant, Tween 80® was used to facititate the solution of o-chlorobenzaldehyde in water. Controls showed no harmful effects from Tween 80® alone.

Damage was assessed one, three, and nine days after treatment, using the following rating scale:

0 - No Damage	3 - Heavy Damage
1 - Slight Damage	4 - Extreme Damage
2 - Moderate	5 - Death

Height measurements were taken nine days after treatment.

TABLE I. QUANTITIES OF CHEMICALS USED FOR PLANT TREATMENTS EXPRESSED AS Ml/2000 Ml WATER

COMPOUND	CONCENTRATION 0	1	2	3	4
Decontaminated CS-2	0	2.500	5.000	10.000	20.000
o-Chorobenzaldehyde	0	0.430[a]	0.860[a]	1.720[a]	3.440[a]
Malononitrile	0	0.240[a]	0.480[a]	0.960[a]	1.920[a]
Monoethanolamine (MEA)	0	0.590[a]	1.080[a]	2.170[a]	4.340[a]
2-Propanol	0	0.630[a]	1.207[a]	2.414[a]	4.828[a]
2-Propanol/MEA	0/0	0.630[a]/ 0.590[a]	1.207[a]/ 1.080	2.414[a]/ 2.170[a]	4.828[a]/ 4.340[a]

[a]Based on the amount of the particular compound that would be found in the quantity of decontaminated CS-2 in the same column.

Mammalian Studies

Adult Long-Evans (Hooded) Rats® (Rattus novegicus Berkenhout) were the test organisms used in the LD_{50} (lethal dose for 50 percent population) determinations. The rats, a minimum of 90 days old, had minimum body weights of 175 grams for males and 200 grams for females. The animals were divided into five groups, (10 individuals per group). Their daily diet consisted of Purina Laboratory Chow.® None were fasted prior to dosage. Survivors of the experiments were held for daily observation until apparent complete recovery or for a maximum of 21 days. Oral dosage (1.25 to 2.25 g/kg of decontaminated CS-2) were administered via stomach tube. Distilled water was used as the solvent or suspending agent for the decontamination products. Dosing was accomplished by using a 2-1/2 cc syringe with 0.1 cc graduations and a blunt-pointed 7.3 cm 16-gauge spinal needle which served as the stomach tube. To prevent injury to the esophagus the blunt end of the needle was built up with parafilm® into a head 0.3 cm in diameter. The tube did not reach the stomach of the rat but was extended into the esophagus far enough to insure receipt of full dosage. The LD_{50} values were determined by the methods of Litchfield and Wilcoxon(2) and Reed and Muench.(3)

To evaluate hazards associated with decontaminated CS-2 in water sources, Swiss Webster mice (Mus musculus L.) were introduced to CS-2 decontamination products in their water supply. The mice, ranging in weight from 20 to 35 grams were housed in groups of four. Concentration of CS-2 decontamination products in their water supply was 500 ppm; therefore, each mouse predictably would ingest from 60 to 130 mg/kg each day. Three generations of these mice were subjected to the same conditions for a period of 90 days.

Fish Studies

Acute TL_m (median tolerance limit) evaluations were performed in accordance with the Routine Bioassay Method.(4)

The test organisms, mosquitofish (Gambusia affinis Baird and Girard) and bluegill (Lepomis macrochirus Raf.), were seined from lakes on Eglin Reservation, Florida. The mosquitofish, total length 20 to 30 mm, and bluegill, total length 40 to 60 mm, were acclimatized in the laboratory in rectangular 10 gallon holding tanks for a minimum of 10 days before they were used. Water temperature in holding tanks and test containers was maintained at 22 C. Test animals were fed Longlife Pool Fish Food® daily. None were fasted prior to testing.

Test containers were cylindrical 4.5 gallon laboratory glass jars (FSN 6640-430-1240), housing 10 fish. Water temperature was maintained at 22 C and water depth at 250 mm. Between tests, containers were washed with detergent and rinsed with acetone. Test animals were observed hourly for the first eight hours and at 24 hour intervals thereafter throughout the 96 hours of observation.

TL_m values were determined by the method of Reed and Muench.(3)

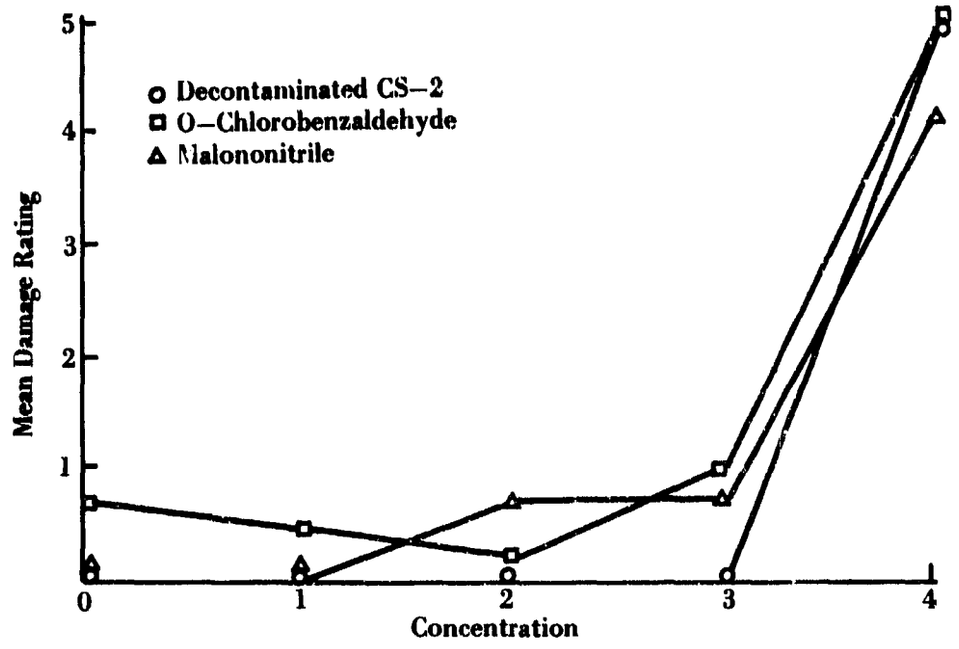

Figure 1. Mean Damage to Rice Nine Days After Treatment With Decontaminated CS-2 and Its Hydrolysis Products

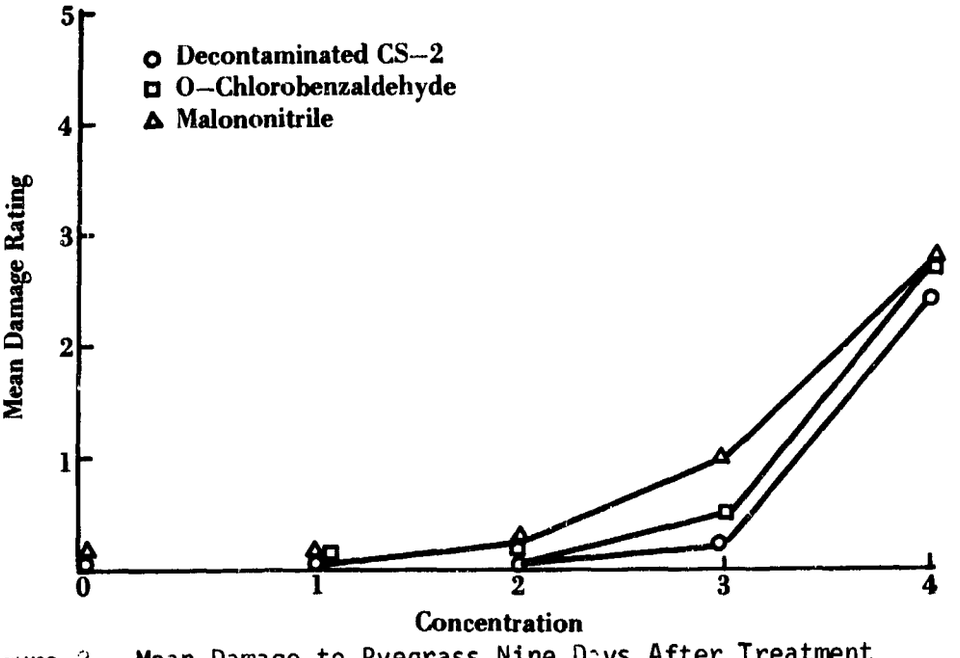

Figure 2. Mean Damage to Ryegrass Nine Days After Treatment With Decontaminated CS-2 and Its Hydrolysis Products

SECTION III

TEST RESULTS AND DISCUSSION

Plant Studies

At the concentrations used in this experiment, applications of MEA, 2-propanol, or a mixture of these two compounds resulted in no damage to the treated plants. Decontaminated CS-2 and equivalent amounts of o-chlorobenzaldehyde and malononitrile produced damage with varying concentration levels. Within 24 hours following treatment, slight damage was evident on plants which had received concentration 4 of the three solutions. After three days, concentration 4 had produced moderate to heavy damage while concentrations 1, 2, and 3 still showed little or no effect. By the ninth day further damage was noted with concentration 4, and concentrations 1, 2, and 3 had produced slight to moderate damage.

Bleaching of leaf pigments, flaccidity of stems and leaves, and a reduction in plant height were general symptoms exhibited by the damaged plants. Because of leaf damage (symptomatic of a potassium deficiency) noted in tomato plants prior to treatment, statistical data for this species were not obtained. However, the raw data provides an indication of tomato responses. Rice and ryegrass responded similarly to decontaminated CS-2 and its hydrolysis products. At the lower concentrations, however, o-chlorobenzaldehyde caused significantly more damage to cotton than decontaminated CS-2 or malononitrile. An increase in damage to all species appears to occur between concentrations 3 and 4 (Figures 1 through 4).

Plants treated with o-chlorobenzaldehyde exhibited variable responses. Examination of the mean damage scores for this treatment indicated that cotton (2.4) was more susceptible than rice (0.9) or ryegrass (0.6). Raw data indicated that tomato plants were more tolerant to o-chlorobenzaldehyde. Responses of all species to malononitrile were similar.

Results from preliminary investigations indicated that one.percent concentrations of decontaminated CS-2 would cause death in the test species. However, in this experiment, few death ratings were assigned to any species. The variations in results could be indicative that during the decontamination process, the levels of products formed changed with time. [5,6] These changes could have caused the variation in phytotoxic properties of decontaminated CS-2. Due to variations in

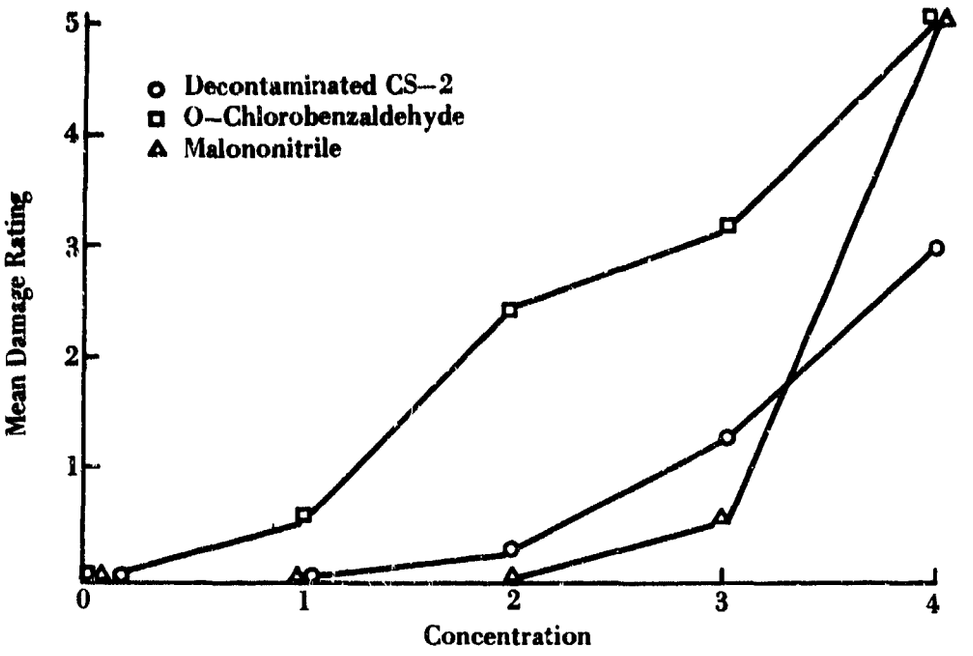

Figure 3. Mean Damage to Cotton Nine Days after Treatment with Decontaminated CS-2 and Its Hydrolysis Products.

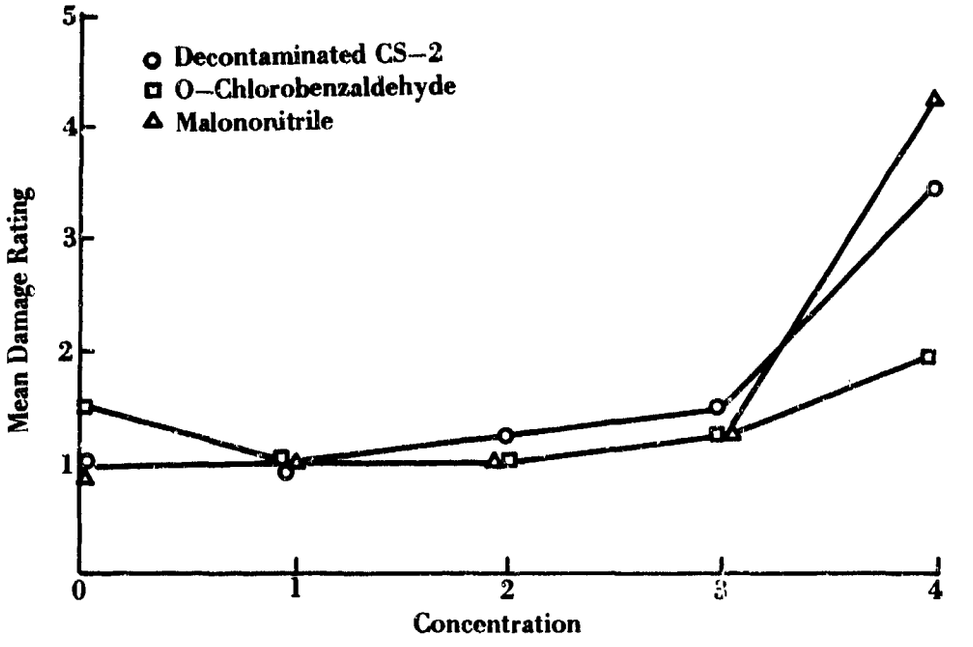

Figure 4. Mean Damage to Tomatoes Nine Days after Treatment with Decontaminated CS-2 and Its Hydrolysis Products.

results, an additional study was performed, using two percent solutions of decontaminated CS-2 and equivalent amounts of its hydrolysis products. In this study, all plants which received these concentrations were dead after nine days. No statistical analyses were performed for this study.

Mammalian Studies

For Long-Evans (Hooded) Rats,® the acute oral LD_{50} of CS-2 decontamination products was established at 1.68 g/kg by the Litchfield-Wilcoxon method[2] and 1.69 g/kg by the Reed-Muench method[3] (Figures 5 and 6).

The Swiss Webster mice which were subjected to CS-2 decontamination products in their water supply exhibited no visible deleterious effects. No deaths occurred in the test groups during the 90-day observation period. During three consecutive generations of parent exposure to decontaminated CS-2, there were no detectable abnormalities found in the offspring.

Fish Studies

Using the methods of Reed and Muench,[3] THE TL_m values for decontaminated CS-2, malononitrile, o-chlorobenzaldehyde, 2-propanol and monoethanolamine were determined for mosquitofish and bluegill. For 96 hour exposure periods, the TL_m values for decontaminated CS-2 were 18.95 ppm for mosquitofish and 16.78 ppm for bluegill. The values for malononitrile were 1.80 ppm for mosquitofish and 4.25 ppm for bluegill. For o-chlorobenzaldehyde, the values were 1.80 ppm for mosquitofish and 0.57 ppm for bluegill. TL_m values for 2-propanol were greater than 1,400 ppm for both fish species. For monoethanolamine, the values were 337.5 ppm for mosquitofish and 329.16 ppm for bluegill (Tables II and III).

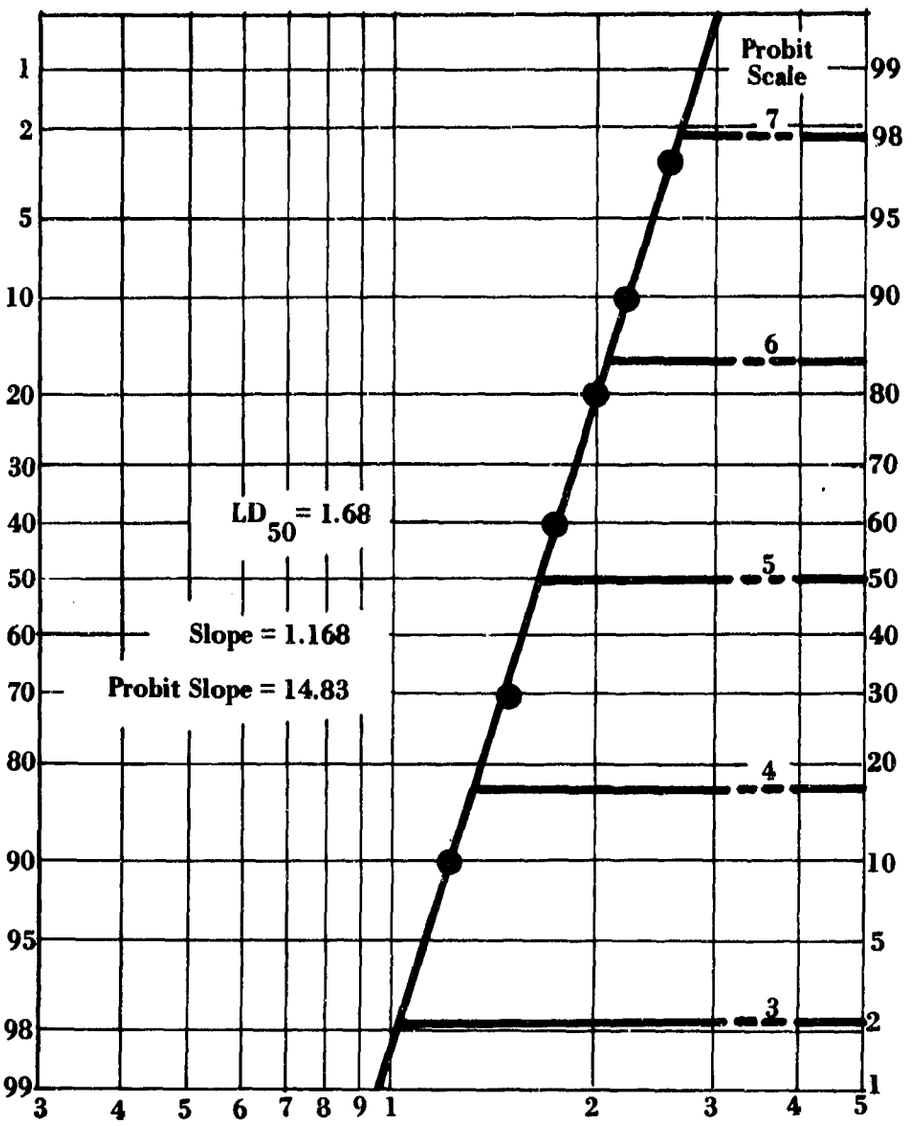

Figure 5. LD_{50} Reaction Probability of Decontaminated CS-2 for Rats (Litchfield-Wilcoxon Method).

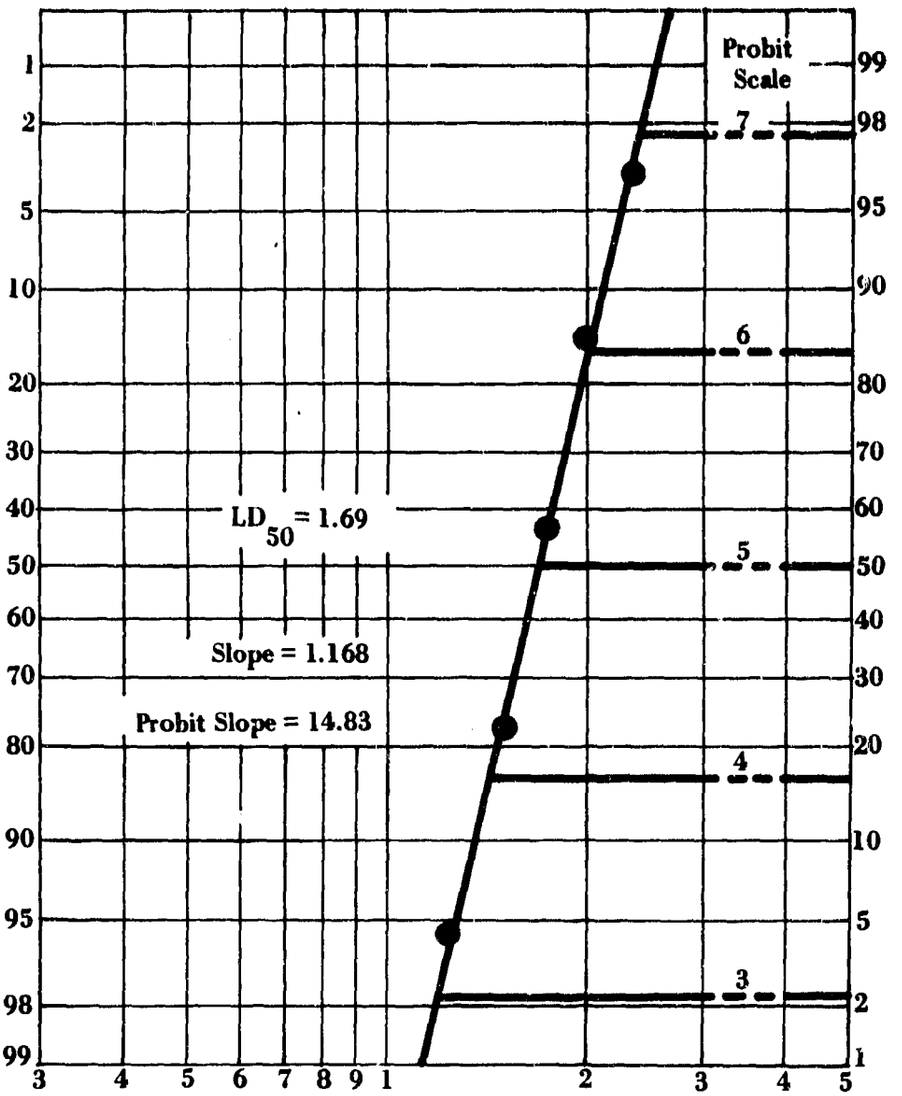

Figure 6. LD_{50} Reaction Probability of Decontaminated CS-2 for Rats (Reed-Muench Method).

TABLE II. TL_m VALUES FOR MOSQUITOFISH (IN PPM) MAINTAINED IN SOLUTIONS OF CS DECONTAMINANT COMPONENTS, DECONTAMINATED CS-2 AND CS-2 HYDROLYSIS PRODUCTS.

COMPOUND	EXPOSURE TIME IN HOURS			
	24	48	72	96
Monoethanolamine (MEA)	375.00	360.41	350.00	337.5
Malononitrile	> 2.60	2.52	1.95	1.80
o-Chlorobenzaldehyde	8.90	8.90	8.90	8.90
2-Propanol	> 1400.00	> 1400.00	> 1400.00	> 1400.00
Decontaminated CS-2	> 30.00	26.78	22.22	18.95

TABLE III. TL_m VALUES FOR BLUEGILL (IN PPM) MAINTAINED IN SOLUTIONS OF CS DECONTAMINANT COMPONENTS, DECONTAMINATED CS-2 AND CS-2 HYDROLYSIS PRODUCTS.

COMPOUND	EXPOSURE TIME IN HOURS			
	24	48	72	96
Monethanolamine (MEA)	> 375.00	365.90	345.90	329.16
Malononitrile	0.77	0.71	0.64	0.57
o-Chlorobenzaldehyde	4.50	4.33	4.33	4.25
2-Propanol	> 1400.00	> 1400.00	> 1400.00	> 1400.00
Decontaminated CS-2	> 25.00	> 25.00	24.00	16.78

SECTION IV

CONCLUSIONS

Experimental data indicate that the toxicity of decontaminated CS-2 to plants and mammals is sufficiently low so as not to pose serious problems in disposing of the neutralized riot control agent. However, the toxicity of decontaminated CS-2 to fish is high enough to warrant caution in its disposal near streams or lakes.

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13. ABSTRACT

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Monoethanolamine - Water Decontaminating Solution						
2-Propanol - Water Wetting Solution						
Fish Toxicity						
Plants Toxicity						
Mammalian Toxicity						
CS-2 Decontamination Products						
Surfactant						